Investigating the Interactions of Bisphosphonates with Amyloid Beta (Aβ) Proteins in Alzheimer's Disease

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- **Abstract:** The unmet therapeutic need for a devastating neurodegenerative disorder, Alzheimer's disease (AD), which is marked by the deposition of beta-amyloid (Aβ) plaques in the brain – mandates the development of novel anti-Aβ therapies. We investigated the **drug re-repurposing** potential of **bisphosphonates (BPs)** class of drugs as anti-Aβ agents. It is known that women are more prone to get AD and are also observed to suffer from osteoporosis after menopause. Therefore, we determined the interactions of BPs with Aβ proteins. The anti-Aβ-aggregation activity against Aβ40 peptide was evaluated by conducting fluorescence aggregation kinetic studies, transmission electron microscopy (TEM), and computational modeling. Preliminary results suggested that BPs exhibit anti-Aβ activity. Risedronate and alendronate were identified as promising inhibitors of Aβ aggregation. Molecular docking studies for dimer model of Aβ40 peptide indicated that both risedronate and alendronate showed interactions in the aggregation-prone region of the dimer peptide model. These studies demonstrate that BPs exhibit anti-Aβ activity in vitro and can be used to discover and develop novel anti-AD agents.
- **Keywords:** Alzheimer's disease, bisphosphonates, drug repurposing, molecular docking, osteoporosis, dimer model of Aβ40 peptide, anti-Aβ agents
- **Background:** Repositioning of existing drugs is regarded as a strategic approach to reveal novel therapeutic uses for already pre-existing drugs. This is an effective strategy that can provide known drugs with established safety and tolerance drug profiles, which reduces the financial burden that arises during the development stages. This potential approach thus decreases the time length and ardous journey involved in bringing forth a new drug into the market. The pathological similarity shared by AD and osteoporosis, furthermore, provides the opportunity to investigate the repurposing of BPs in reducing and or treating the incidence of AD in postmenopausal women and discover new therapies. In this regard, we conducted *in silico* investigation of FDA approved bisphosphonate drugs; a library of 6 osteoclast inhibitors or antiresorptive agents – etidronate, alendronate, pamidronate, ibandronate, risedronate and zoledronic acid, Fig. 1, using the dimer model of Aβ40. We further explored the inhibition and/or modulation activity of BPs towards the Aβ40 peptide using a thioflavin T (ThT) – based fluorescence assay to identify bisphosphonates with promising anti-Aβ activity.

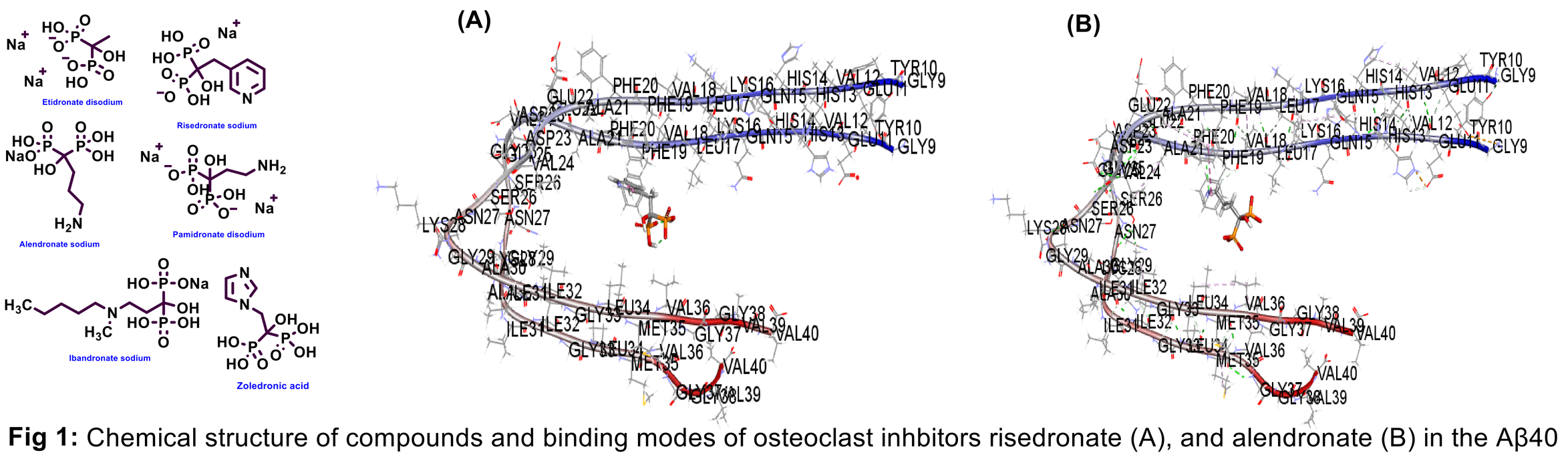

Fig 1: Chemical structure of compounds and binding modes of osteoclast inhbitors risedronate (A), and alendronate (B) in the Aβ40 dimer model.

Table 1: CDOCKER Energy and CDOCKER Interaction Energy data for osteoclast inhibitors in the Aβ40 dimer model

Compound Name	CDOCKER Energy in Kcal/mol	CDOCKER Interaction Energy in Kcal/mol
Risedronate	–25.27	– 15.13
Alendronate	–14.61	– 14.84

The CDOCKER energy and CDOCKER interaction energies for the top ranked binding poses of bisphosphonates obtained after conducting molecular docking studies on the Aβ40 dimer model (PDB ID: 2LMN using the CDOCKER algorithm in the software *Discovery Studio Structure-Based-Design*, BIOVIA Inc)

Table 2: Percentage inhibition of Aβ40 aggregation at 37°C at the end of 24 h at 25 µM

Compound	%Inhibition ± SD
Etidronate	17 ± 3
Pamidronate	43.2 ± 3
Alendronate	49.7 ± 22
Ibandronate	34.8 ± 13
Risedronate	68.7 ± 18
Methylene blue	99.3 ± 14
Resveratrol	92.7 ± 25

Results are expressed as average ± SD (n=2-3)

Results: Our *in silico* studies identified two bisphosphonate drugs that have the potential to interact and bind to Aβ40 dimer model. Furthermore, our molecular docking studies show these two bisphosphonate class of drugs can undergo favourable interactions in the aggregation prone region (KLVFFA) of Aβ40 dimer model. In vitro fluorescence aggregation kinetics study demonstrated that risedronate exhibits effective inhibition of Aβ40 aggregation at 25 µM.

Conclusions: This study shows that bisphosphonate derivatives exhibit anti-Aβ activity, suggesting that these templates hold promise and can be chemically modified to design and develop brain penetrating anti-Aβ therapies for AD.

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